XVII. *The relation between the secondary sexual characters and the Gonads and accessory sexual glands in Insects.* By E. A. Cockayne, M.A., D.M., F.R.C.P.

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The remarkable effects of the secretions of the ductless glands on the development of the secondary sexual characters in the vertebrates makes it of great interest to examine the evidence for and against the existence of a similar relationship in insects. In mammals and birds we know that in the male the interstitial cells, or cells of Leydig, which lie between the seminiferous tubules of the testis, produce a secretion which causes the normal development of the secondary sexual characters in the male at puberty. Removal of the testes before puberty prevents their appearance, but atrophy of the seminiferous tubules in no way affects them. The testis is a double organ, each part having its own distinct function. In the ovary a similar state of affairs appears to exist, but has not been so clearly demonstrated.

Besides this comparatively simple relationship between the interstitial glandular part of the gonads and the secondary sexual characters, the ductless glands exert an influence upon one another. For instance, over-activity of the cortex of the suprarenal gland produces sexual precocity, under-activity of the pineal body produces a similar effect; but under-activity of the pituitary body delays the appearance of secondary sexual characters. These stimulating or restraining effects seem to be due to the action of the internal secretions of these glands on the gonads, causing an increase or decrease in the secretion of the interstitial cells, and so only indirectly hastening or retarding the appearance of secondary sexual characters.

But it must be remembered that long before these cells become active we find well-marked differences in the two sexes, and such differences still remain if the cells be removed and are present even in cases where the gonads have never developed at all. It is only some of the more striking differences, which appear at the time of puberty, and which are due to the activity of the gonads.

The short account of the action of the ductless glands given above is that most commonly accepted as correct, but it must be mentioned that Blair Bell and others consider that they all have an equally powerful influence on the development of the secondary sexual characters, and that, although alike in appearance in the two sexes, the pituitary, suprarenal, and other glands of the male and female, produce internal secretions as unlike as those of the ovary and testis themselves.

It is important to examine what evidence there is for and against the existence of a similar control in insects. The best evidence is afforded by—

(1) Experimental castration and transplantation of the gonads in Lepidoptera.

(2) Temperature experiments in Lepidoptera.

(3) Stylopisation in Hymenoptera.

(4) Gynandromorphs and Intersexes.

Castration experiments have been performed by Oudemans, Kellogg and Meisenheimer.

Oudemans removed the testes or ovaries from larvae of *Lymantria dispar* before the penultimate and last moults; thirty out of sixty survived. Castrated males copulated and castrated females tried to lay, but merely deposited the tuft of wool with which the normal females cover their eggs; their external appearance was unaffected.

Kellogg experimented on silkworms, *Bombyx mori*, and in no case was any change in the secondary sexual characters produced.

Meisenheimer castrated 600 larvae of *Lymantria dispar* and bred 186 imagines. Those operated on before the second moult always died, but some survived which were operated on between the second and third, and third and fourth moults. None of the imagines were altered in appearance.

Transplantation of the ovaries or testes into larvae of which the testes or ovaries had been removed also failed to alter their secondary sexual characters; nor was any effect produced by forming artificial hermaphrodites, though the transplanted gonads grew.

These experiments are against the existence of a relationship between the gonads and secondary sexual characters such as is found in vertebrates.

By means of heat or cold acting for varying periods on the pupae of certain Lepidoptera the females can be made

to assume in some degree the colour and structure characteristic of the males, and the males, though to a less extent, can be made to approach the females in structure and colour.

Merrifield by means of heat obtained females of *Gonepteryx rhamni* having the yellow colour of the males, and Standfuss obtained females of *Perisomena* (*Saturnia*) *caecigena* with feathered antennae, though these are simple in the normal female. Kosminsky by subjecting pupae of *Lymantria dispar* to cold produced in some males an alteration of the colour and shape of the scales towards those found in the females, and in females he produced a colour nearly as dark as that of the males, a shape in the scales very like that found in the opposite sex, and shorter feathered instead of longer simple antennae. The testes and ovaries were smaller or unaltered in size, and the ova were always infertile. He considered that the maldevelopment of the sex-glands was not associated with the alteration in colour and structure, because those with the most normal glands were sometimes those with most marked alteration in secondary sexual characters.

The parasite *Stylops* in the bees *Andrena labialis* and *chrysosceles* may produce in some females an approach to the male in colour and to a smaller extent in structure, and in some males a similar but less marked approach to the female. In other specimens no effect is produced. The ovaries are reduced to about one-quarter their normal size. In other bees no effect on the secondary sexual characters is produced, though the ovaries are equally reduced in size. These observations, published by Geoffrey Smith and Hamm, like the temperature experiments, fail to show a direct relationship between the gonads and the secondary sexual characters.

The conditions found in gynandromorphs afford us still more definite evidence. In halved gynandromorphs there is perfect development of the secondary sexual characters of the male on one side and of the female on the other; yet the most varied arrangement of gonads and accessory glands are met with. In the normal male there are two testes and glandulae accessoriae; in the female two ovaries, two cement-glands and the receptaculum seminis or spermatheca, probably also a secretory gland. In gynandromorphs secondary sexual characters of the male may be perfectly developed not only in the absence of the testes

and glandulae accessoriae, but also in the presence of one or two ovaries, one or two cement-glands and the spermatheca. Similarly the secondary sexual characters of the female may be normally and fully developed in the absence of ovaries, cement-glands and spermatheca and in the presence of the gonads and accessory glands of the male. Thus the presence of gonads and glands of the one sex has no modifying influence on the secondary sexual characters of the other sex, even in the absence of the gonads and glands proper to it. In some cases where gonads and accessory glands of both sexes are present the secondary sexual characters of both sexes are also present—male characters on the side of the male organs and female on the side of the female organs. The gynandromorph *Amorpha hybridus* described in the preceding paper also proves that secondary sexual characters of both sexes may be present, male on one side, female on the other, though neither testis nor ovary be present. Indeed, in this case the accessory glands of both sexes were also absent with the exception of a distorted cement-gland.

The ducts, vasa deferentia, vesiculae seminales, and ductus ejaculatorius in the male, oviducts, vagina, bursa copulatrix and cervix in the female, can also be completely absent without affecting the development of the secondary sexual characters, so that it is evident that their epithelium produces no secretion which influences the development of the secondary sexual characters.

In the peculiar gynandromorphous *Agriades coridon* var. *roystonensis*, of which I have published descriptions in the Journal of Genetics and in these Transactions, scales of a structure peculiar to the male were found usually on the wings of one side only, yet no male internal organs were present, and in most instances the female organs were perfectly formed. The ovaries in all cases were of equal size and fully developed.

In some primary hybrid hawk-moths of the genus *Amorpha*, of which a list has been published recently by Dannenberg, and of which *A. ocellatus* ♂ and *A. populi* ♀ and *A. ocellatus* ♂ × *A. austati* ♀ are the best known, females are replaced by insects in which the external genitalia are roughly symmetrical, but show a mosaic of male and female characters. The male and female parts are for the most part fully formed, and are not intermediate in characters between the two sexes. Ill-formed

ovaries and female accessory glands are present, but no male internal organs are found. Other primary hybrids occasionally show a similar condition. On the other hand, a primary hybrid *Ennomos* bred by Harrison showed fully-developed male organs including two testes, and yet parts of the external genitalia and some external somatic characters were purely female in appearance.

Many secondary hybrid *Saturnias* (emperor moths) and *Bistoninae* show a very coarse mosaic of male and female somatic characters, including the external genitalia, though their internal organs are entirely female.

The secondary hybrid *Amorpha daubi*, Standfuss, *A. ocellatus* ♂ × *A. langi* ♀, also has females replaced by gynandromorphs or intersexes. This is also the case with many mongrel *Lymantrias*, though in these the external genitalia are intermediate between those normally found in the two sexes. Here, again, we have several examples of male secondary sexual characters where all the male internal organs are absent and all the female present, and one example of the opposite condition.

The chief argument against the castration experiments is that they can only be performed comparatively late in the larval stage, and it has been suggested that at this stage an internal secretion has been produced and already acted on the tissues of the body in such a way that they are able to attain to their full sexual differentiation, even after the gonads have been removed. Such an argument cannot be advanced in the case of halved gynandromorphs in which the failure of development of the sexual organs when it occurs must take place at a much earlier period, and still less can it be advanced in the case of the hybrid gynandromorphs or "intersexes."

The whole of the evidence derived from experimental castration, and from the study of halved gynandromorphs and of intersexes, seems to prove definitely that the secondary sexual characters are in no way dependent on the gonads or any other portion of the internal sexual apparatus. There is, however, no proof to be derived from them that some gland quite independent of the sexual organs does not produce an internal secretion comparable to that of the interstitial cells of the vertebrate testis and ovary.

But if we examine the condition of affairs in mixed and transverse gynandromorphs even this possibility seems

to be excluded. Gynandromorphs are occasionally met with in which the head or head and thorax show fully-developed male characters and the rest of the insect shows female characters. In others exactly the opposite condition is met with.

When the whole of the external structure of the head or thorax shows male characters it is almost certain that the structures within are male in constitution also.

If this be admitted one can exclude any part of the head, thorax or abdomen as the possible site of a gland producing an internal secretion, which causes the development of the secondary sexual characters in insects.

Thus we are reduced either to accept the view that in insects the tissues are sexually differentiated from the first, and do not need the stimulus of any internal secretion in order to attain the fullest development; whereas, in vertebrates, although the tissues are sexually differentiated from the beginning, the differentiation cannot be completed without the influence of the internal secretion of the gonads and other glands.

Or we must accept an alternative explanation such as that of Geoffrey Smith, that there is in all animals a sexual formative substance, which in some is capable alone of perfecting the sexual characters, but which in others requires the co-operation of the internal secretion of the gonads. It is difficult to accept the former hypothesis even in the case of insects, because the parasite *Stylops* in bees and subjection to abnormally high or low temperatures in butterflies and moths can in some instances modify the secondary sexual characters at a late stage of development.

But on the latter hypothesis, unless the evidence derived from a study of gynandromorphism be untrustworthy, the sexual formative substance must be produced in various parts of the body if not by all the tissues.

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